

DATA EVALUATION RECORD
FRESHWATER SEDIMENT *Chironomus riparius* EMERGENCE TEST

1. **CHEMICAL:** Pyraclostrobin PC Code: 099100

2. **TEST MATERIAL:** BAS 500 F Purity: 100%

3. **CITATION:**

Authors: Kuhl, R., and V. Wydra

Title: Effects of BAS 500 F (Pyraclostrobin) on the Development of Sediment Swelling Larvae of *Chironomus riparius* in a Sediment-Water System – Exposed via Spiked Sediment.

Study Completion Date: March 6, 2013

Laboratory: Institut fuer Biologische Analytik und Consulting IBACON GmbH
Arheilger Weg 17
64380 Rossdorf, Germany

Sponsor: BASF SE
67056 Ludwigshafen, Germany

Laboratory Report ID: 407435

MRID No.: 49080401

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CSS-Dynamac Corporation

Signature: 

Date: 06/24/13

APPROVED BY: John Marton, Ph.D., Environmental Scientist, CDM Smith

Signature: 

Date: 11/08/13

5. **APPROVED BY:** Meghan Radtke, Ph.D., Biologist

Signature: 

Date: 12/9/13

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Chironomus riparius*

Age of Test Organism: 1st instar larvae, 3 days post-hatch

Definitive Test Duration: 28 days

Study Method: Static, with aeration

Type of Concentrations: Sediment, pore and overlying water mean-measured

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7. **CONCLUSIONS:**

Results Synopsis:

Based on mean-measured sediment concentrations:

EC₅₀: >2.82 mg ai/kg dw 95% C.I.: N/A

28-day NOAEC: 2.82 mg ai/kg dw

28-day LOAEC: >2.82 mg ai/kg dw

Based on mean-measured pore water concentrations:

EC₅₀: >10.8 µg ai/L 95% C.I.: N/A

28-day NOAEC: 10.8 µg ai/L

28-day LOAEC: >10.8 µg ai/L

Based on mean-measured overlying water concentrations:

EC₅₀: >22.1 µg ai/L 95% C.I.: N/A

28-day NOAEC: 22.1 µg ai/L

28-day LOAEC: >22.1 µg ai/L

Assessment endpoints: emergence rate and development rate

Endpoints affected: none*

*The reviewer's comparison of treated levels to the negative control only revealed no significant differences (p>0.05); however, the study authors' comparison to the pooled control showed a reduction in emergence rate at the highest treated level.

8. **ADEQUACY OF THE STUDY:**

A. Classification: Supplemental

B. Rationale: This study followed methods described in OECD Guideline 218 (adopted 2004): "Sediment-Water Chironomid Toxicity Test Using Spiked Sediment", and does not fulfill any current U.S. EPA data requirement.

C. Reparability: N/A

9. **MAJOR GUIDELINE DEVIATIONS (from OECD Guideline 218):**

For development rate, the sensitivity of the sexes was not assessed.

10. **SUBMISSION PURPOSE:** RS

11. MATERIALS AND METHODS

Stability of Compound Under Test Conditions: Overlying water, pore water, and sediment samples from the negative control, solvent control, 1.2 and 4.8 mg ai/kg sediment levels were analyzed at 0 and 28 Days. The analytical results of the sediment samples showed that over time there was a significant decrease of the test item in the sediment, while results of the water samples indicated that the pore water concentrations were higher than the overlying water concentrations at the test start, and <LOQ in almost all water samples at test termination.

Concentrations of BAS 500 F (pyraclostrobin) in the test system.

Nominal Initial Conc. Applied to Sediment (mg ai/kg dw)	Day	Sediment		Pore Water, µg ai/L	Overlying Water, µg ai/L
		mg ai/kg dw	% Rec.		
Control	0, 28	<LOQ ^(a)	---	<LOQ	<LOQ
Solvent Control	0, 28	<LOQ	---	<LOQ	<LOQ
1.2	0	1.03	86	5.20 ^(b)	<LOQ
	28	<LOQ	---	<LOQ	Not detected
4.8	0	4.83	101	43.73	20.34
	28	0.81	17	<LOQ	1.19

(a) The LOQ was 1-1.83 µg ai/L for aqueous samples and 0.57 mg ai/kg dw for sediment samples.

(b) One replicate sample measured 9.48 µg ai/L, while the second replicate sample measured <LOQ; the value shown here is the average of the first sample and ½ the LOQ.

Physicochemical properties of pyraclostrobin.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound.

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
<u>Species</u> <i>Chironomus riparius</i>	<i>Chironomus riparius</i> (Meigen)
<u>Source</u>	Laboratory-bred
<u>Culture Conditions</u> A reproduction and oviposit chamber should consist of an adult area, sufficiently large to allow swarming (minimum 30 x 30 x 30 cm), and an oviposit area. Crystallizing dishes or larger containers with a thin layer of quartz sand (5 to 10 mm) or Kieselgur (thin layer to a few mm) spread over the bottom and containing suitable water to a depth of several cm are suitable as an oviposit area. Environmental conditions: temperature 20±2°C; 16:8 hours light:dark (intensity ca. 1000 lux); air humidity ca. 60%	Cultures were maintained at 20 ± 2°C in the same water used for testing. Further culturing details (with the exception of feeding) were not provided.
<u>Egg Mass Acclimation Period</u> Four to five days before test initiation freshly laid egg masses should be taken from cultures and maintained separately in culture medium, temperature change should not exceed 2°C per day.	Fresh egg masses were isolated from the main culture 5 days prior to study initiation and placed in small vessels filled with culture medium. The larvae began to hatch 2 days after the eggs were laid, and were 3 days old at study initiation.
<u>Age of Test Larvae</u> First instar (1 to 4 days post-hatch with confirmation)	1 st instar, 3 days post-hatch
<u>Food</u> Green algae (e.g., <i>Scenedesmus subspicatus</i> , <i>Chlorella vulgaris</i>) or flaked fish food as a ground powder, suspension, or filtrate	Hatched chironomus larvae were fed <i>ad libitum</i> with TetraMin® commercial fish diet.
<u>Health of parent culture stock</u> Were parent chironomids in good health during the culture period?	Not reported.

B. Test System

Guideline Criteria	Reported Information
<p><u>Test Material</u></p>	<p>Identity: BAS 500 F (pyraclostrobin) IUPAC name: methyl 2-[1-(4-chlorophenyl)pyrazol-3-yloxymethyl]-<i>N</i>-methoxycarbanilate CAS name: methyl <i>N</i>-[2-[[[1-(4-chlorophenyl)-1<i>H</i>-pyrazol-3-yl]oxy]methyl]-phenyl]-<i>N</i>-methoxycarbamate CAS No.: 175013-18-0 Batch No.: 10-510009 Description: yellow to yellow-brown solid Purity: 100.0% Storage: +5 to +30°C</p>
<p><u>Type of Test System</u></p> <p>Static (static-renewal or flow-through of overlying water is evaluated on a chemical-specific basis). Distilled or deionized water may be added to overlying water once daily as needed to maintain volume.</p>	<p>Static with aeration. Three times during the study (due to evaporation), test vessels were refilled with de-ionized water. It was reported that water levels did not change by more than 10% during the study.</p> <p>Additional test vessels were prepared for chemical analysis of the test item on Day 0, and biological replicates were used for chemical analysis on Day 28. Therefore, the method for analytical sampling did not affect volume, biological load, or test concentration.</p>
<p><u>Test Water</u></p> <p>Soft reconstituted water or water from a natural source is preferred. Dechlorinated tap water may be used if the test organism will survive in it for the duration of the culturing and testing without showing signs of stress.</p>	<p>Reconstituted water (Elendt M4 medium) was prepared with de-ionized water and analytical-grade salts; a detailed chemical composition was provided.</p> <p><u>Characteristics of the M4 medium:</u> Total hardness: 249.2 mg/L as CaCO₃ Alkalinity: 0.9 mmol/L Conductivity: 588 µS/cm pH: 7.9 Oxygen saturation: 100% (8.8 mg/L)</p>

Guideline Criteria	Reported Information
<p><u>Test Sediment</u> Formulated (reconstituted, artificial, or synthetic) sediment is recommended. Content of sediment by dry weight: 5% peat (dry) (pH 5.5-6.0) or alpha-cellulose, 75% quartz sand (>50% in size range of 50-200 microns), 20% kaolinite clay (kaolinite content ca. 30%), CaCO₃ 0.05-0.1%). Moisture content 30-50%, TOC 2% (±0.5%) and pH 6.5 - 7.5. Natural sediment can be used if it is fully characterized, unpolluted, and free of organisms that might compete with or consume chironomids. (If solvent other than water will be used, sand content of artificial sediment is adjusted accordingly.)</p>	<p>Formulated sediment was prepared on a dry weight basis using the following components: 4.5% sphagnum moss peat (pH 5.0-6.0), 76% quartz sand (>50% with a particle size of 50-200 µm), 20% kaolinite clay (kaolinite content of ≥30%), 0.7% calcium carbonate (to adjust the pH) and 2200 mL de-ionized water.</p> <p>The peat was air-dried and ground to a fine powder. The dry ingredients were combined and the pH adjusted with calcium carbonate. Then, de-ionized water was added.</p> <p><u>Sediment characterization:</u> TOC: 2.2% Moisture content: 38.6% pH: 7.3</p>
<p><u>Sediment Spiking</u></p>	<p>A 20-mL aliquot of the appropriate dosing solution (in acetone) was applied to 90 g dry quartz sand, and the solvent was allowed to completely evaporate. The spiked sand was then added to 810 g (dw) formulated sediment (total of 900 g dw) and the spiked sediments were thoroughly mixed to homogeneity.</p>
<p><u>Sediment Conditioning</u> <u>Artificial sediment:</u> 7 days in flowing dilution water prior to test initiation, chambers may be aerated</p>	<p>Test systems (spiked-sediment:overlying water) were prepared 2 days prior to introduction of the test organisms and allowed to acclimate under test conditions; during this time, the systems were gently aerated.</p>

Guideline Criteria	Reported Information
<u>Introduction of Test Organisms</u> Twenty-four hours prior to test initiation aeration of chambers is stopped and organisms are added to the chambers. Aeration should not resume for at least 24 hours. At test initiation, the test substance is spiked into the overlying water column.	At test initiation (Day 0), chironomid larvae were randomly added to each replicate test vessel. Aeration was stopped while the animals were added and for approximately 24 hours thereafter.
<u>Solvents</u> If used, minimal (i.e., #0.1 ml/l) and same concentration in all treatments. Suitable solvents are acetone, ethanol, methanol, ethylene glycol monoethyl ether, ethylene glycol dimethyl ether, dimethylformamide or triethylene glycol. (OECD guidelines also allows use of dispersants: Cremophor RH40, Tween 80, methycellulose 0.01%, and HCO-40)	Acetone, 20 mL per 900 g (dw) sediment The solvent was allowed to completely evaporate following the spiking procedure.
<u>Water Temperature</u> 20°C ± 2°C (Should not deviate between vessels by more than 1°C.)	20 to 21°C
<u>pH</u> <u>Sediment</u> : 7.0 ± 0.5 <u>Interstitial Water</u> : <u>Overlying Water</u> : 6.0 to 9.0 (Should not vary by more than 1 unit during test)	<u>Sediment</u> : 7.3 (at preparation) <u>Interstitial Water</u> : Not determined <u>Overlying Water</u> : 7.7 to 8.6
<u>TOC</u> <u>Sediment</u> : 2 ± 0.5% <u>Overlying Water</u> : 2 mg/L	<u>Sediment</u> : 2.2% (at preparation) <u>Overlying Water</u> : Not determined
<u>Ammonia</u> <u>Interstitial Water</u> : <u>Overlying Water</u> :	<u>Interstitial Water</u> : Not determined <u>Overlying Water</u> : 1.2 to 2.0 mg/L

Guideline Criteria	Reported Information
<p><u>Total Water Hardness</u> 200 mg/L as CaCO₃ (prefer 160 to 180 mg/L as CaCO₃)</p>	<p>284.8 to 329.3 mg/L as CaCO₃ (OECD 218 specifies that total hardness not exceed 400 mg/L as CaCO₃)</p>
<p><u>Dissolved Oxygen</u> 60% air saturation value throughout test</p>	<p>62 to 100% saturation</p>
<p><u>Aeration</u> Aeration (ca. one bubble/sec) is allowed except for when larvae are being added and for at least 24 hours after introduction of test organisms to a test chamber. If one test chamber is aerated all test chambers must be treated the same.</p>	<p>Test vessels were gently aerated (not further specified).</p>
<p><u>Test Vessels or Compartments</u> 1. <u>Material</u>: Glass, No. 316 stainless steel, teflon or perfluorocarbon plastics 2. <u>Size</u>: Sediment depth of 1.5- 3 cm and the depth ratio of sediment to water should be ca. 1:4, must not be >1:4; 600 ml beaker with 8 cm diameter</p>	<p><u>Material</u>: glass beakers <u>Size</u>: 600 mL; 1.6-cm layer of sediment (100 ± 1 g wet weight) and 6.4-cm Elendt M4 medium depth (280 ± 10 mL). The height ratio was 1:4 sediment to overlying water.</p>
<p><u>Covers</u> Test vessels should be covered with a glass plate.</p>	<p>Test vessels were covered with glass plates, minimizing evaporation.</p>
<p><u>Photoperiod</u> 16 hours light, 8 hours dark (Light intensity 500 to 1000 lux)</p>	<p>16 hours light, 8 hours dark Light intensity 620 to 720 lux</p>
<p><u>Food</u> Green algae (e.g., <i>Scenedesmus subspicatus</i>, <i>Chlorella vulgaris</i>) or flaked fish food as a ground powder, suspension, or filtrate</p>	<p>Finely-ground Tetramin® fish food</p>

Guideline Criteria	Reported Information
<p><u>Food Concentration and Frequency</u> Preferably feed daily but at least 3 times per week. <u>day 1 to 10:</u> 0.25-0.5 mg per larvae per day <u>remainder of test:</u> 0.5-1 mg per larvae per day (keep to a minimum, should not accumulate on sediment surface, cause overlying water to be cloudy or cause drop in DO)</p>	<p>Daily</p> <p>Days 0 to 10: 0.5 mg per larvae per day Days 11 to 27: 0.5 to 1.0 mg per larvae per day</p>

C. Test Design

Guideline Criteria	Reported Information
<p><u>Duration</u> <i>Chironomus riparius</i>: 28 days (if midges emerge early the test can be terminated after a minimum of 5 days after emergence of the last adult in the control).</p>	<p>28 days</p>
<p><u>Nominal Concentrations</u> Negative control, solvent control (if a solvent was used) and at least 5 test concentrations. (Note exception to dilution factors described below can be made for shallow slope responses but minimum number of test concentrations may need to be increased)</p> <p><u>ECx endpoint</u>: test concentrations should bracket ECx and span the environmental concentration range. Dilution factor should not be greater than two between exposure concentrations.</p> <p><u>NOEC/LOEC endpoint</u>: factor between concentrations must not be greater than 3.</p>	<p>Negative control, solvent control, 0.3, 0.6, 1.2, 2.4 and 4.8 mg ai/kg dw sediment</p> <p><u>ECx endpoint</u>: A nominal dilution factor rate of 2 was used.</p> <p><u>NOAEC/LOAEC endpoint</u>: A nominal dilution factor rate of 2 was used.</p>

Guideline Criteria	Reported Information
<p><u>Number of Test Organisms**</u></p> <p><u>ECx endpoint</u>: 60 larvae per treatment level; 3 replicates per treatment level</p> <p><u>NOAEC/LOAEC endpoint</u>: at least 80 larvae per treatment level with at least 4 replicates per treatment level (adequate power to detect a 20% difference, Type I error rate 5%)</p> <p>**(Optional) If data on 10-day growth and survival are needed additional replicates (number based on ECx or NOEC/LOEC endpoint determination) should be included at test initiation.</p>	<p><u>ECx endpoint</u>: Generally 80 larvae per treatment level with 4 replicates per treatment level. The solvent control group maintained six replicates, for a total of 120 larvae.</p> <p><u>NOAEC/LOAEC endpoint</u>: Generally 80 larvae per treatment level with 4 replicates per treatment level. The solvent control group maintained six replicates, for a total of 120 larvae.</p> <p>**(Optional) 10-day growth data were not collected.</p>
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Organisms were randomly assigned to test containers.</p>
<p><u>Overlying Water Parameter Measurements</u></p> <ol style="list-style-type: none"> 1. Dissolved oxygen should be measured daily in all test chambers. 2. Temperature and pH should be measured in all test chambers at the start and end of the test and at least once a week during the test. 3. Temperature should be monitored at least hourly throughout the test in one test chamber. 4. Hardness and ammonia should be measured in the controls and one test chamber at the highest concentration at the start and end of the test. 	<ol style="list-style-type: none"> 1. – 2. At the start and end of the test and once per week during the test in each vessel. 3. Not conducted; criteria not required in OECD 218 guidance. 4. At the start and end of the test in a pooled sample of the control replicates, the replicates of the solvent control and the replicates of the highest test concentration.

Guideline Criteria	Reported Information
<u>Chemical Analysis-Overlying Water</u> At a minimum must be analyzed at test initiation (i.e., one hour after introduction of test substance into the test chamber) and at the end of the test in at least the highest concentration and one lower concentration.	Analyzed on Days 0 and 28 at the control, solvent control, 1.2 and 4.8 mg ai/kg sediment levels
<u>Interstitial Water and Sediment Isolation Method</u> Centrifugation (e.g., 10,000 g and 4 EC for 30 min) is recommended. If test substance is demonstrated not to adsorb to filters, filtration may be acceptable.	Centrifugation at 4000 rpm for 30 minutes
<u>Chemical Analysis-Interstitial Water</u> At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration.	Analyzed on Days 0 and 28 at the control, solvent control, 1.2 and 4.8 mg ai/kg sediment levels
<u>Chemical Analysis-Bulk Sediment</u> At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration.	Analyzed on Days 0 and 28 at the control, solvent control, 1.2 and 4.8 mg ai/kg sediment levels

12. **REPORTED RESULTS**

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in compliance with the most recent edition of the OECD Principles of GLP; German Chemicals Act (ChemG), Annex 1; and EEC Directive 2004/10/EC.
<u>Control Mortality</u> <30%	Yes

Guideline Criteria	Reported Information
Did chironomids emerge in controls between day 12 and 23?	Negative control – Days 15 to 23 Solvent control – Days 16 to 24
<u>Control Emergence</u> Mean emergence between 50-70%	Negative control – 78.8% emergence Solvent control – 71.7% emergence
<u>Data Endpoints</u> <u>Emergence Test (28 day)</u> <ul style="list-style-type: none"> - Number alive - Time to emergence - Number of emerged male and female midges - Number of visible pupae that have failed to emerge - Number of egg masses deposited - Observations of other effects, abnormal behavior, or appearance or clinical signs (e.g., leaving sediment, unusual swimming) <u>Growth and Survival (10-day) (Optional)</u> <ul style="list-style-type: none"> - Number alive - Instar level of surviving larvae - Dry weight (ash free) per test chamber of surviving larvae by instar level 	<u>Emergence Test (28 day)</u> <ul style="list-style-type: none"> - Number alive - Time to emergence - Number of emerged male and female midges - Number of visible pupae that have failed to emerge -Number of egg masses deposited - Observations of other effects, abnormal behavior, or appearance or clinical signs (e.g., leaving sediment, unusual swimming) <u>Growth and Survival (10-day) (Optional)</u> N/A
Raw data included?	Yes, sufficient

Effects Data

Table 1. Summary of BAS 500 F (Pyraclostrobin) effects on *Chironomus riparius* emergence success and sex ratio

Toxicant Concentration				Initial No.	Mean Number Emerged			Mean Sex Ratio ^(b) (%)		Emergence (% ± SD)
Nominal Sediment (mg ai/kg dw)	Mean Measured ^(a)				♂	♀	Total	ER ♂	ER ♀	
	Sediment (mg ai/kg)	Overlying Water (µg ai/L)	Pore Water (µg ai/L)							
Negative control	<LOQ	<LOQ	<LOQ	80	37	26	63	59	41	78.8 ± 19.7
Solvent control	<LOQ	<LOQ	<LOQ	120	39	47	86	45	55	71.7 ± 11.7
0.3	N/A	N/A	N/A	80	27	26	53	51	49	66.3 ± 13.1
0.6	N/A	N/A	N/A	80	31	29	60	52	48	75.0 ± 4.1
1.2	0.66	<LOQ	2.85	80	35	24	59	59	41	73.8 ± 12.5
2.4	N/A	N/A	N/A	80	36	25	61	59	41	76.3 ± 9.5
4.8	2.82	10.8	22.1	80	27	21	48	56	44	60.0 ± 10.8*

^(a) The LOQ was 1-1.83 µg ai/L for aqueous samples and 0.57 mg ai/kg dw for sediment samples.

^(b) ER_♂ = number of emerged males/number of emerged larvae; ER_♀ = number of emerged females/number of emerged larvae; reviewer-calculated and reported as percentages.

* Significant difference in emergence rate from the pooled control (α=0.05).

Table 2. Summary of BAS 500 F (Pyraclostrobin) effects on *Chironomus riparius* development time and rate.

Toxicant Concentration				Days to First Emergence ^(b)	Mean Development Rate ^(c) (1/day ± SD)
Nominal Sediment (mg ai/kg dw)	Mean Measured ^(a)				
	Sediment (mg ai/kg)	Overlying Water (µg ai/L)	Pore Water (µg ai/L)		
Negative control	<LOQ	<LOQ	<LOQ	15	0.057 ± 0.001
Solvent control	<LOQ	<LOQ	<LOQ	16	0.055 ± 0.001
0.3	N/A	N/A	N/A	16	0.057 ± 0.003
0.6	N/A	N/A	N/A	15	0.056 ± 0.001
1.2	0.66	<LOQ	2.85	15	0.056 ± 0.002
2.4	N/A	N/A	N/A	14	0.059 ± 0.003
4.8	2.82	10.8	22.1	16	0.058 ± 0.002

^(a) The LOQ was 1-1.83 µg ai/L for aqueous samples and 0.57 mg ai/kg dw for sediment samples.

^(b) Reviewer-determined from summarized data tables. Does not represent mean days to first emergence.

^(c) Mean development rate = $\sum_{i=1}^m \frac{f_i x_i}{n_e}$

where: i = index of inspection interval; m = maximum number of inspection intervals; f_i = number of midges emerged in the inspection interval i ; n_e = total number of midges emerged; and

$x_i = \frac{1}{\left(\text{day}_i - \frac{l_i}{2}\right)}$ which is the development rate of the midges emerged in interval i ; day_i = inspection day (days since application); and l_i = length of inspection interval i (days, 1 day in this study)

Toxicity Observations: Emergence averaged 78.8 and 71.7% for the negative and solvent control groups, respectively, compared to 66.3, 75.0, 73.8, 76.3 and 60.0% for the nominal 0.30, 0.60, 1.2, 2.4, and 4.8 mg ai/kg sediment treatment levels, respectively. The difference was statistically-significant ($\alpha = 0.05$) compared to the pooled control (75.2%) at the 4.8 mg ai/kg level. The subsequent NOAEC and LOAEC for emergence rate were 2.4 and 4.8 mg ai/kg, respectively. The 28-day EC_{50} was >4.8 mg ai/kg.

Emergence commenced on Days 14 to 16 for all levels, with no treatment-related effect evident.

Development rates ranged from 0.056 to 0.059 days⁻¹ for all control and treatment levels, with no statistically-significant differences indicated at any level compared to the pooled control (0.056 days⁻¹). The subsequent NOAEC and LOAEC for development rate were 4.8 and >4.8 mg ai/kg, respectively. The 28-day EC_{50} was >4.8 mg ai/kg.

It was reported that no egg masses were deposited in the test vessels prior to study termination, and that no visible pupae failed to emerge.

B. Statistical Results (From Study Report)

Emergence rate and development rate were statistically analyzed using pooled sexes. Analyses were performed using ToxRat statistical software and reported in terms of initial sediment concentrations.

For both endpoints, the negative and solvent control data were compared using a Student t-test for homogeneous variances ($p < 0.05$); no significant differences were observed, and the data were pooled for subsequent comparisons. Data were assessed for normality using Shapiro-Wilk's test and for homogeneity of variances using Levene's test ($p < 0.05$). The data for both parameters met both assumptions and were subsequently analyzed using ANOVA and Williams one-sided t-test ($p < 0.05$). Treatment-level data were compared to the performance of the pooled controls.

The EC_{50} values were determined to be greater than the highest concentration tested for both endpoints.

Most sensitive endpoint: emergence rate

Endpoint	Methods	EC ₅₀ (95% CI) (mg ai/kg dw)	NOAEC (mg ai/kg dw)	LOAEC (mg ai/kg dw)
28-d Emergence Rate	Williams t-test	>4.8	2.4	4.8
28-d Development Rate	Williams t-test	>4.8	4.8	>4.8
10-d Survival (Optional)	---	---	---	---
10-d Growth (Optional)	---	---	---	---

13. VERIFICATION OF STATISTICAL RESULTS

Summary of Statistical Methods used for NOAEC/LOAEC Analyses.

Endpoint	Solvent vs Dilution Control		NOAEC/LOAEC	
	Method	Diff ⁽¹⁾ (%)	Method	Diff ⁽²⁾ (%)
28-d Emergence Rate	Two-Sample T	9.88	Dunnett/ Visual	3.17
28-d Development Rate- Males	Two-Sample T	2.67	Dunnett	-5.12
28-d Development Rate- Females	Two-Sample T	0.72	Dunnett	-3.61
Male:Female	Two-Sample T	1.2	Dunnett	15.5
10-d Survival (Optional)	--	--	--	--
10-day Dry Weight (Optional)	--	--	--	--

⁽¹⁾ Difference between the mean dilution water and solvent control responses.

⁽²⁾ Difference between the dilution water and NOAEC concentration treatment.

Most sensitive endpoint: None

Verification Statistical Endpoint Values^(a) (mg ai/kg dw).

Statistical Endpoint	28-day Emergence	28-day Male Development Rate	28-day Female Development Rate	Male:Female
NOAEC	4.8	4.8	4.8	4.8
LOAEC	>4.8	>4.8	>4.8	>4.8
IC ₅₀ (95% C.I.)	>4.8	>4.8	>4.8	>4.8
Slope (Standard Error)	N/A	N/A	N/A	N/A

^(a) Results are based on nominal test concentrations.

14. REVIEWER'S COMMENTS:

The reviewer's analysis for % emergence on Day 28 did not detect a significant reduction at the highest level, while the study authors' did. This difference is likely due to the fact that the reviewer compared treatment data to the negative control only, whereas the study author compared treatment data to the pooled control. All analyses were conducted using CETIS version 1.8.7.12 statistical software with backend database settings implemented by EFED on 31 May 2013. Results were reported in terms of mean-measured sediment, pore and overlying water concentrations.

The study was designed to fulfill OECD Guideline 218 "Sediment-Water Chironomid Toxicity Test Using Spiked Sediment (2004), and was conducted in accordance with the GLP Principles of the OECD and of the German "Chemikaliengesetz" (Chemicals Act). This study does not fulfill any current U.S. EPA guideline requirement. One notable deviation from guidance was observed: the sensitivity between sexes was not assessed for development rate data. This deviation does not affect the scientific soundness of this study. All guideline validity requirements were fulfilled.

Fortified samples were prepared and run concurrently with the test samples. Recoveries of pyraclostrobin from fortified water samples was 97% (n=22, CV=7%) and from fortified sediment samples was 94% (n=10, CV=12%).

For sample analyses, aliquots of the overlying and isolated pore water were diluted with

acetonitrile and filtered through a PTFE membrane filter (0.45- μ m) prior to analysis using LC/MS/MS. Sediment pellets were extracted with acetone by shaking for 30 minutes. Samples were centrifuged (4000 rpm for 5 minutes) and filtered through a PTFE membrane filter (0.45- μ m), and an aliquot of the extract was evaporated to dryness. Residues were re-dissolved in acetonitrile:dilution water (80:20, v:v) into the calibration curve and analyzed using LC/MS/MS.

The mean-measured concentration reported for the nominal 2.4 mg ai/kg sediment level (i.e., 1.37 mg ai/kg dw) was determined through extrapolation of the lower and higher mean-measured concentrations.

The experimental study dates were July 25 to December 11, 2012.

15. REFERENCES:

Analytical Method L0166/01 Determination of BAS 500 F, M500F01, M500F02 and M500F07 in Soil at LOQ 0.001 mg/kg, BASF SE Agricultural Center Limburgerhof Crop Protection Division Ecology and Environmental Analytics, April 2010.

Chemikaliengesetz der Bundesrepublik Deutschland (ChemG), Anhang 1, in der Fassung der Bekanntmachung vom 2. juli 2008 (BGBl. I S. 1146), zuletzt geandert durch Art. 5 Abs. 39 G v, 24. Februar 2012 (BGBl. I S. 212)

DIN 32645:1994-05, Chemical analysis – Decision limit, detection limit and determination limit under repeatability conditions – Terms, methods, evaluation.

Directive 2004/10/EC of 11 February 2004 amending Council Directive 87/18/EEC, Official Journal of the European Union No. L 50:44-59.

OECD Guidelines for the Testing of Chemicals, “Sediment-Water Chironomid Toxicity Test Using Spiked Sediment”, OECD Guideline 218, adopted April 13, 2004.

OECD Principles of Good Laboratory Practice, adopted by Council of 26th November 1997 [C(97)186/Final], Environment Directorate, Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17, Paris 1998.

APPENDIX I. MEAN-MEASURED CONCENTRATION CALCULATIONS:

Sediment			
Nominal Conc.	Day	Measured	
mg ai/kg		mg ai/kg	
1.2	0	0.998	
		1.054	
	28	0.285	*
		0.285	*
	Mean	0.66	
4.8	0	5.081	
		4.578	
	28	0.936	
		0.689	
	Mean	2.82	
Overlying Water			
Nominal Conc.	Day	Measured	
mg ai/kg		ug ai/L	
1.2	0	<LOQ	
		<LOQ	
	28	<LOQ	
		<LOQ	
	Mean	<LOQ	
4.8	0	20.298	
		20.378	
	28	1.055	
		1.322	
	Mean	10.76	

Pore Water			
Nominal Conc.	Day	Measured	
mg ai/kg		ug ai/L	
1.2	0	9.476	
		0.915	*
	28	0.5	*
		0.5	*
	Mean	2.85	
4.8	0	41.146	
		46.318	
	28	0.5	*
		0.5	*
	Mean	22.12	
*Measured results were <LOQ, and thus 1/2 the appropriate LOQ was used for calculation purposes.			

CETIS Summary Report

Report Date: 29 Oct-13 11:39 (p 1 of 3)
 Test Code: 099100 49080401 | 17-2986-4076

OPPTS 850.1735 Sub-Chronic Sediment (10-d FW)

IBACON

Batch ID: 20-1644-7893	Test Type: Chronic Sediment Chironomus	Analyst:
Start Date: 25 Jul-12	Protocol: OPPTS 850.1735 Sub-chronic Sediment (1	Diluent: Elendt's M4 Medium
Ending Date:	Species: Chironomus dilutus	Brine: Not Applicable
Duration: NA	Source: Lab In-House Culture	Age: 3day

Sample ID: 02-9351-9028	Code: 49080401	Client: CDM Smith
Sample Date: 25 Jul-12	Material: Pyraclostrobin	Project: Fungicide
Receive Date:	Source: BASF Corporation	
Sample Age: NA	Station:	

Batch Note: PC Code 099100 MRID 49080401

Sample Note: PC Code 099100 MRID 49080401

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
01-7980-8980	Female Development Rate	0	>0		4.13%		Equal Variance t Two-Sample Test
00-6404-9461	Female Development Rate	4.8	>4.8	NA	8.48%		Dunnett Multiple Comparison Test
09-3547-6517	Male Development Rate	0	>0		5.39%		Equal Variance t Two-Sample Test
15-3372-1186	Male Development Rate	4.8	>4.8	NA	6.15%		Dunnett Multiple Comparison Test
06-0671-1526	Male to Female Ratio	0	>0		74.1%		Equal Variance t Two-Sample Test
09-7424-7016	Male to Female Ratio	4.8	>4.8	NA	109.0%		Dunnett Multiple Comparison Test
08-9961-2912	Percent Emerged	0	>0		28.8%		Equal Variance t Two-Sample Test
06-4074-4661	Percent Emerged	4.8	>4.8	NA	27.1%		Dunnett Multiple Comparison Test

CETIS Summary Report

Report Date: 29 Oct-13 11:39 (p 2 of 3)
Test Code: 099100 49080401 | 17-2986-4076

OPPTS 850.1735 Sub-Chronic Sediment (10-d FW)

IBACON

Female Development Rate Summary

C-mg/kg sed	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	6	0.105	0.102	0.107	0.103	0.109	0.000957	0.00235	2.24%	0.0%
0	Negative Control	4	0.104	0.098	0.109	0.101	0.109	0.0018	0.00359	3.46%	0.72%
0.3		4	0.105	0.0914	0.119	0.0989	0.118	0.00434	0.00868	8.25%	-0.69%
0.6		4	0.104	0.0996	0.108	0.101	0.106	0.00131	0.00263	2.53%	0.72%
1.2		4	0.105	0.0968	0.114	0.101	0.113	0.00266	0.00532	5.05%	-0.72%
2.4		4	0.105	0.0974	0.113	0.0992	0.109	0.00241	0.00482	4.59%	-0.53%
4.8		4	0.108	0.102	0.113	0.103	0.111	0.00185	0.0037	3.44%	-2.87%

Male Development Rate Summary

C-mg/kg sed	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	6	0.109	0.105	0.114	0.103	0.114	0.00165	0.00403	3.69%	0.0%
0	Negative Control	4	0.112	0.106	0.119	0.107	0.117	0.00206	0.00411	3.66%	-2.67%
0.3		4	0.114	0.109	0.119	0.111	0.118	0.00149	0.00299	2.63%	-4.04%
0.6		4	0.116	0.109	0.122	0.113	0.122	0.0021	0.00419	3.62%	-5.87%
1.2		4	0.113	0.106	0.121	0.109	0.12	0.00239	0.00479	4.23%	-3.58%
2.4		4	0.12	0.112	0.127	0.117	0.127	0.00243	0.00486	4.06%	-9.53%
4.8		4	0.118	0.113	0.123	0.115	0.121	0.00147	0.00294	2.49%	-7.93%

Male to Female Ratio Summary

C-mg/kg sed	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	6	0.882	0.528	1.24	0.4	1.4	0.138	0.337	38.2%	0.0%
0	Negative Control	4	1.75	-0.406	3.91	0.73	3.75	0.678	1.36	77.4%	-98.8%
0.3		4	1.19	0.177	2.21	0.44	2	0.319	0.638	53.5%	-35.3%
0.6		4	1.36	-0.269	2.99	0.45	2.75	0.513	1.03	75.2%	-54.5%
1.2		4	1.84	-0.303	3.98	0.57	3	0.673	1.35	73.2%	-108.0%
2.4		4	1.87	-0.434	4.16	0.8	4	0.723	1.45	77.5%	-112.0%
4.8		4	1.48	0.561	2.4	0.67	2	0.289	0.577	39.0%	-67.9%

Percent Emerged Summary

C-mg/kg sed	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	6	0.717	0.594	0.839	0.6	0.9	0.0477	0.117	16.3%	0.0%
0	Negative Control	4	0.788	0.473	1	0.55	0.95	0.0987	0.197	25.1%	-9.88%
0.3		4	0.663	0.453	0.872	0.55	0.85	0.0657	0.131	19.8%	7.56%
0.6		4	0.75	0.685	0.815	0.7	0.8	0.0204	0.0408	5.44%	-4.65%
1.2		4	0.738	0.539	0.936	0.55	0.8	0.0625	0.125	16.9%	-2.91%
2.4		4	0.763	0.612	0.913	0.7	0.9	0.0473	0.0946	12.4%	-6.4%
4.8		4	0.6	0.428	0.772	0.5	0.75	0.054	0.108	18.0%	16.3%

CETIS Summary Report

Report Date: 29 Oct-13 11:39 (p 3 of 3)

Test Code: 099100 49080401 | 17-2986-4076

OPPTS 850.1735 Sub-Chronic Sediment (10-d FW)

IBACON

Female Development Rate Detail

C-mg/kg sed	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Solvent Blank	0.103	0.103	0.105	0.103	0.109	0.104
0	Negative Control	0.109	0.101	0.103	0.102		
0.3		0.118	0.101	0.0989	0.103		
0.6		0.101	0.106	0.102	0.106		
1.2		0.113	0.104	0.101	0.103		
2.4		0.109	0.103	0.0992	0.109		
4.8		0.106	0.111	0.11	0.103		

Male Development Rate Detail

C-mg/kg sed	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Solvent Blank	0.109	0.107	0.113	0.11	0.114	0.103
0	Negative Control	0.112	0.113	0.107	0.117		
0.3		0.118	0.113	0.111	0.113		
0.6		0.122	0.114	0.113	0.114		
1.2		0.12	0.111	0.109	0.113		
2.4		0.127	0.118	0.117	0.117		
4.8		0.121	0.12	0.116	0.115		

Male to Female Ratio Detail

C-mg/kg sed	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Solvent Blank	0.78	1.4	0.71	1	1	0.4
0	Negative Control	1.2	3.75	0.73	1.33		
0.3		1.2	0.44	2	1.13		
0.6		0.45	1.5	0.75	2.75		
1.2		0.57	3	3	0.78		
2.4		4	0.8	1.33	1.33		
4.8		1.75	1.5	2	0.67		

Percent Emerged Detail

C-mg/kg sed	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Solvent Blank	0.8	0.6	0.6	0.9	0.7	0.7
0	Negative Control	0.55	0.95	0.95	0.7		
0.3		0.55	0.65	0.6	0.85		
0.6		0.8	0.75	0.7	0.75		
1.2		0.55	0.8	0.8	0.8		
2.4		0.75	0.9	0.7	0.7		
4.8		0.55	0.5	0.6	0.75		